

# Thymoquinone Ameliorates Oxidative Stress and Inflammatory Responses and Protects against Monosodium Iodoacetate-Induced Osteoarthritis in Rats

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## ABSTRACT

**Background:** Osteoarthritis is a degenerative joint condition defined by the destruction of cartilage, resulting in pain, stiffness, and impaired joint function. There is a growing need for safe and effective therapies that can address the underlying causes of osteoarthritis and slow its development. **Objectives:** This study assessed the efficacy of thymoquinone in ameliorating experimentally induced osteoarthritis in rats and investigated the mechanisms by which thymoquinone influences osteoarthritis. **Materials and Methods:** Osteoarthritis was induced in rats by administering Monosodium Iodoacetate (MIA). The experimental rats received thymoquinone treatment starting one week before to MIA induction and continued for four weeks. The pain response and weight-bearing level of rats were evaluated after completion of treatments. The concentrations of biochemical markers, oxidative stress-related biomarkers, inflammatory cytokines, Prostaglandin E2 (PGE-2), and caspase-3/9 levels were evaluated in the experimental rats. Furthermore, the levels of Nrf2, HO1, Cyclooxygenase-2 (COX-2), and NF- $\kappa$ B were assessed using kits. **Results:** The findings of this work indicated that thymoquinone administration effectively increased the paw withdrawal latency and threshold, and regulated the biochemical marker levels in the MIA-induced rats. Thymoquinone treatment considerably decreased oxidative stress via increasing antioxidants, reduced inflammatory cytokine levels, PGE-2, and caspase-3/9 levels in MIA-induced rats. Moreover, the thymoquinone effectively increased the Nrf2/HO1 levels, decreased COX-2, and NF- $\kappa$ B levels in the MIA-induced rats. **Conclusion:** The present findings evidenced that the thymoquinone mitigated the osteoarthritis-related pathological alterations induced by MIA in rats by modulating inflammation and oxidative stress-related biomarker levels. These data demonstrate that thymoquinone successfully ameliorated MIA-induced osteoarthritis in rats.

**Keywords:** Nrf2/HO1 Pathway, Thymoquinone, Osteoarthritis, Inflammation, Cyclooxygenase-2.

## INTRODUCTION

Osteoarthritis is a widespread chronic degenerative joint disease defined by joint pain and stiffness, significantly diminishing the quality of life for affected individuals. The condition is marked by the progressive destruction of articular cartilage, subchondral bone remodeling, and synovial inflammation.<sup>1</sup> It is not merely a process of wear and tear but an active process involving intricate molecular and biochemical changes within the entire joint

structure. The initial understanding of osteoarthritis as solely an age-related ailment stemming from biomechanical factors has evolved substantially.<sup>2</sup> Current perspectives recognize it as a multifaceted condition influenced by genetic predispositions, metabolic abnormalities, inflammatory mediators, and mechanical stressors. Osteoarthritis is a major contributor to disability and morbidity worldwide, leading to substantial personal, social, and economic burdens. The increasing global prevalence underscores the critical need for effective strategies in disease management and prevention. The prevalence of knee osteoarthritis in adults can reach as high as 40% among individuals aged 70-74.<sup>3</sup> Projections reveal a substantial increase in osteoarthritis incidences in the coming decades, posing a significant challenge to healthcare systems globally. These trends emphasize the importance of understanding and addressing the



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risk factors associated with osteoarthritis to mitigate its escalating impact on society.<sup>4</sup>

The development of osteoarthritis is multifaceted, encompassing a complex interaction of genetic, biomechanical, and biochemical factors. Genetic factors contribute significantly to osteoarthritis susceptibility, with certain gene polymorphisms influencing cartilage structure and function. Mechanical stress, whether from acute joint injury or chronic overuse, can initiate or accelerate the degenerative process. Obesity is a significant risk factor, influencing local conditions through heightened joint stress and systemic conditions via generation of adipokines and pro-inflammatory markers.<sup>5</sup> Systemic factors, encompassing metabolic and inflammatory conditions, are recognized to play a crucial role in the onset and advancement of osteoarthritis. A history of joint trauma, repetitive joint use, underlying anatomical disorders, and previous joint infections also contribute to osteoarthritis development. The intricate web of etiological factors highlights the need for personalized preventive and therapeutic interventions targeting modifiable risk factors to alleviate the burden of osteoarthritis.<sup>6</sup>

The pathophysiology of osteoarthritis involves a cascade of events leading to the destruction of the articular cartilage and changes in the underlying bone and synovium. Chondrocytes are responsible for maintaining cartilage homeostasis and often undergo phenotypic changes and produce an imbalance of anabolic and catabolic factors. This imbalance can result in the destruction of the extracellular matrix, leading to cartilage thinning and eventual joint space narrowing.<sup>7</sup> The subchondral bone responds to cartilage damage by increasing bone turnover, leading to sclerosis and the formation of osteophytes. Inflammatory factors contribute to cartilage degradation and pain sensitization. The synovium becomes inflamed, leading to synovitis and further production of inflammatory mediators. Understanding the intricate interplay of these processes is critical for advancing targeted therapies to slow disease progression and alleviate symptoms.<sup>8</sup>

Current therapeutic interventions encompass a spectrum of options, ranging from patient education and lifestyle modifications to pharmacological interventions, physical therapies, and surgical procedures. Pharmacological interventions typically include analgesics, nonsteroidal anti-inflammatory drugs, and, in some cases, intra-articular injections of corticosteroids or hyaluronic acid.<sup>9</sup> While these medications can provide symptomatic relief, they often come with a range of side effects, including gastrointestinal complications, cardiovascular risks, and potential cartilage damage with prolonged use. Subsequently, there is a growing need for safe and effective therapies that can address the underlying causes of osteoarthritis and slow its progression.<sup>10</sup> Thymoquinone is a major phytochemical derived from black seed (*Nigella sativa*) and has shown to have various biological activities. It is an essential constituent of black seed oil and has been investigated for

its possible medicinal benefits in several illnesses. Thymoquinone already showed a better anti-inflammatory,<sup>11</sup> renoprotective,<sup>12</sup> cardioprotective,<sup>13</sup> anticancer,<sup>14</sup> chemopreventive,<sup>15</sup> antioxidant,<sup>16</sup> and neuroprotective<sup>17</sup> activities. Nonetheless, the potential significance of thymoquinone as an antiarthritic agent remains unexamined, especially for osteoarthritis. Therefore, this study assessed the efficacy of a thymoquinone in ameliorating experimentally-induced osteoarthritis in rats and investigated the mechanisms by which thymoquinone influences osteoarthritis.

## MATERIALS AND METHODS

### Experimental animals

The present study utilized 7-weeks aged healthy male Sprague-Dawley rats. The rats were maintained in a controlled laboratory environment for seven days. The animals were confined in sterile polypropylene cages. Stringent cleanliness protocols were upheld over the research duration, and cages and bedding were regularly replaced. The standard temperature and relative humidity were constantly maintained, and a 12-hr light/dark series was instituted.

### Treatment groups

Following a one-week acclimatization period, rats were randomly distributed into five groups ( $n=6/\text{group}$ ) as given below: Group I: Normal control rats; Group II: Rats with monosodium Iodoacetate (MIA)-induced osteoarthritis; Groups III and IV: Rats with MIA-Induced osteoarthritis+5 and 10 mg/kg of thymoquinone treatment, respectively; Group V: Rats with MIA-induced osteoarthritis+2 mg/kg of indomethacin (standard drug) treatment. MIA injection (3 mg/50  $\mu\text{L}$ ) was administered to induce osteoarthritis in the rats. The MIA injection was delivered via intra-articular injection to the rats. All rats were administered thymoquinone and indomethacin orally starting one week before to the MIA injection and continued for four weeks.<sup>18</sup>

### Analysis of hind paw weight bearing distribution

The weight-bearing tolerance of the experimental rats were evaluated utilizing capacitance tester. Following the development of osteoarthritis, the rats exhibited reduced weight-bearing capacity in their hind paws. To assess weight bearing distribution, rats were positioned in a measurement chamber for 3 sec to ascertain weight-bearing force. The weight distribution ratio of the rats was determined using the subsequent formula:

$$[\text{Weight in right leg}/(\text{weight in right leg} + \text{weight in left leg})] \times 100$$

### Analysis of pain response

Mechanical sensitivity was tested utilizing a dynamic plantar aesthesiometer. The rats were positioned on a metal mesh surface within a chamber at 22°C, and they were permitted a 15-min acclimatization period prior to the commencement of tests. The tactile stimulator component was positioned beneath

the animal. Upon activation of the aesthesiometer, a plastic monofilament made contact with the paw in proximal metatarsal area. The filament applied a progressively intensifying force on the plantar surface, commencing under the detection threshold and escalating until the stimulus induced discomfort, prompting the rat to withdraw its paw. The force necessary to elicit a paw withdrawal reaction was automatically recorded and quantified in grams. All aesthesiometry experiments employed a maximum force of 50 g and a ramp speed of 20 sec.

### Analysis of biochemical marker levels

The serum concentrations of glucose, creatinine, total protein, Blood Urea Nitrogen (BUN), globulin, albumin, total bilirubin, Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), and Alanine Transaminase (ALT) in the experimental rats were assessed using an automated biochemistry analyzer (Samsung Electronics Co., Korea).

### Analysis of oxidant and antioxidant marker levels

The serum concentrations of oxidative stress indicators, including Malondialdehyde (MDA), Catalase (CAT), Glutathione Peroxidase (GPx), and Superoxide Dismutase (SOD) was evaluated employing the appropriate test kits. All studies were performed in triplicate using the manufacturer's specifications (Elabscience, USA).

### Analysis of inflammatory cytokines

The serum concentrations of Interleukin (IL)-6, IL-18, IL-1 $\beta$ , and TNF- $\alpha$  in the rats were analyzed using commercial test kits. The tests were conducted using three replicates as per the manufacturer's provided specifications (Abcam, USA).

### Analysis of oxidative stress and inflammation-related biochemical marker levels

The concentrations of Prostaglandin E2 (PGE2) and caspase-3/9 in the serum of rats were tested using commercial assay kits as per the manufacturer's specifications (CusaBio, USA). The levels of Nrf-2, HO-1, Cyclooxygenase-2 (COX-2), and NF- $\kappa$ B in the joint tissue homogenates of the rats were analyzed using commercial diagnostic kits. Each test was conducted in triplicate in as per the manufacturer's recommended methods (MyBioSource, USA).

### Statistical analysis

The statistical tests were performed using GraphPad Prism, and the results from each experiment were portrayed as the mean $\pm$ SD of three replicates. The values are evaluated using one-way ANOVA, and subsequently Tukey's *post hoc* tests were performed, with significance established at  $p < 0.05$ .

## RESULTS

### Effect of thymoquinone on the pain response in the experimental rats

Figure 1 illustrates the paw withdrawal latency and threshold and weight bearing levels of the experimental rats. The current results illustrated a considerable decrease in withdrawal latency and threshold and weight bearing in MIA-treated rats. However, the administration of 5 and 10 mg/kg of thymoquinone significantly increased withdrawal latency and threshold and weight bearing in the osteoporosis rats. The administration of standard drug indomethacin (2 mg/kg) demonstrated results comparable to those of thymoquinone treatment, hence corroborating its effects.

### Effect of thymoquinone on the biochemical markers in the experimental rats

The impact of thymoquinone treatment on the serum concentrations of various biochemical markers were evaluated, with the findings illustrated in Figure 2. The rats with MIA-induced osteoarthritis exhibited slight decrease in glucose, BUN, AST, ALP, and bilirubin levels, while slightly elevated the creatinine, total protein, albumin, ALT, and globulin levels when compared with control. However, the thymoquinone treatment at dosages of 5 and 10 mg/kg effectively regulated these biochemical markers in the MIA-induced rats. The administration indomethacin also efficiently regulated these markers in osteoarthritis-induced rats, underscoring the efficacy of thymoquinone.

### Effect of thymoquinone on the oxidative stress markers in the experimental rats

The present findings indicated that rats subjected to osteoarthritis induction exhibited a considerable elevation in MDA levels, while markedly decreasing CAT, GPx, and SOD concentrations in comparison to control rats (Figure 3). Whereas, the treatment of thymoquinone at dosages of 5 and 10 mg/kg effectively decreased MDA levels while increasing the antioxidant concentrations in the osteoarthritis-induced rats, which evidences its antioxidant effects.

### Effect of thymoquinone on the inflammatory cytokines in the experimental rats

Figure 4 illustrates the concentrations of inflammatory cytokines in the serum of experimental rats. An increase in TNF- $\alpha$ , IL-6, IL-18, and IL-1 $\beta$  concentrations were seen in the serum samples of MIA-treated rats. The 5 and 10 mg/kg of thymoquinone treatment significantly reduced these cytokine concentrations in the serum of rats with osteoarthritis. The indomethacin treatment also effectively decreased these cytokine levels in rats with osteoarthritis, hence corroborating the anti-inflammatory efficiency of thymoquinone.

### Effect of thymoquinone on the PGE2 and caspase-3/9 levels in the experimental rats

Figure 5 illustrates the concentrations of PGE2 and caspase-3/9 in the serum samples of the experimental rats. A significant increase in the concentrations of PGE2 and caspase-3/9 was seen in the serum of MIA-induced rats when compared with control. The serum concentrations of PGE2 and caspase-3/9 were significantly reduced by 5 and 10 mg/kg of thymoquinone in rats with osteoarthritis. The results of thymoquinone were further emphasized by the indomethacin, which is also decreased these markers in rats induced with osteoarthritis.

### Effect of thymoquinone on the Nrf2/HO1, COX-2, and NF- $\kappa$ B levels in the experimental rats

The concentrations of Nrf2/HO1, COX-2, and NF- $\kappa$ B in the joint tissue homogenates of the experimental rats were illustrated in Figure 6. A significantly elevated level of COX-2 and NF- $\kappa$ B and subsequently reduced Nrf2/HO1 levels were observed in the joint tissue homogenates of the rats with MIA-treated osteoarthritis when compared with controls. Nonetheless, the 5 and 10 mg/kg dosages of thymoquinone reduced the COX-2 and NF- $\kappa$ B levels and subsequently elevated the Nrf2/HO1 levels in rats with osteoarthritis (Figure 6). These findings were corroborated by the results of indomethacin, which likewise regulated these marker concentrations in rats with osteoarthritis.

## DISCUSSION

Osteoarthritis presents a substantial global health challenge, leading to disability, diminished quality of life, and significant healthcare costs. The complex nature of osteoarthritis stems from

its multifaceted etiology, encompassing genetic predisposition, biomechanical factors, inflammation, and metabolic abnormalities.<sup>19</sup> The failure to achieve substantial progress in elucidating the intricacies of joint microarchitecture and the underlying molecular mechanisms has posed a significant impediment to the creation of viable therapeutic options for the early detection, prevention, and management of osteoarthritis. The pathophysiology of osteoarthritis involves a complex interplay of biomechanical, inflammatory, and biochemical factors that disrupt the homeostasis of the joint environment.<sup>20</sup> Inflammatory mediators, including cytokines and chemokines, contribute to cartilage destruction and synovial inflammation, maintaining the cycle of joint injury. The intricate signaling pathways involving chondrocytes, synoviocytes, and immune cells further contribute to the complexity of osteoarthritis.<sup>21</sup>

The clinical manifestations of osteoarthritis are diverse and vary depending on the affected joint, the stage of the disease, and individual patient factors. Pain is the predominant symptom, frequently characterized as a profound, agonizing sensation that exacerbates with activity and alleviates with rest. The pain associated with osteoarthritis significantly impacts patients' functional abilities, limiting their ability to perform daily tasks like walking, climbing stairs, and grasping objects.<sup>22</sup> This study indicated that rats with osteoarthritis exhibited a decreased paw withdrawal latency and threshold. Interestingly, the thymoquinone treatment increased the withdrawal latency and threshold in the osteoarthritis rats.

Oxidative stress, resulting from a disproportion between the accumulation of ROS and the antioxidant actions, has emerged as

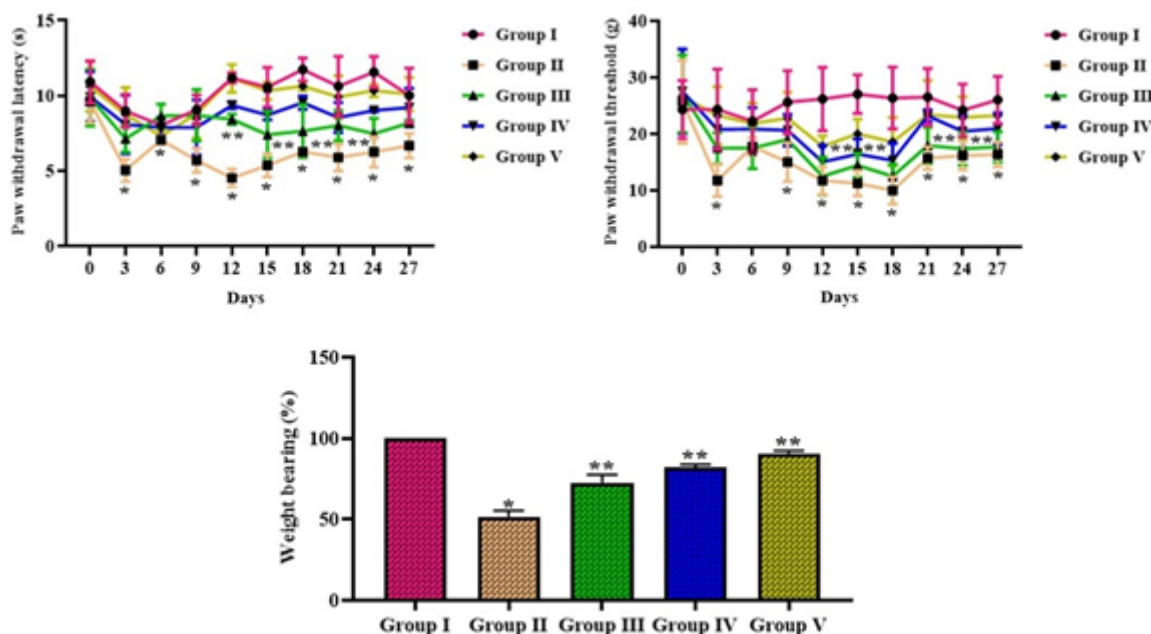


**Figure 1:** Effect of thymoquinone on the pain response in the experimental rats. The data are shown as a Mean $\pm$ SD of three replicate measurements. The values are subjected to statistical analysis by one-way ANOVA and Tukey's *post hoc* tests using Graphpad Prism. Note: '\*' specifies that the values are significant at  $p < 0.01$  in comparison to the control group; '\*\*' specifies that the values are significant at  $p < 0.05$  in comparison to the osteoarthritis-induced group.

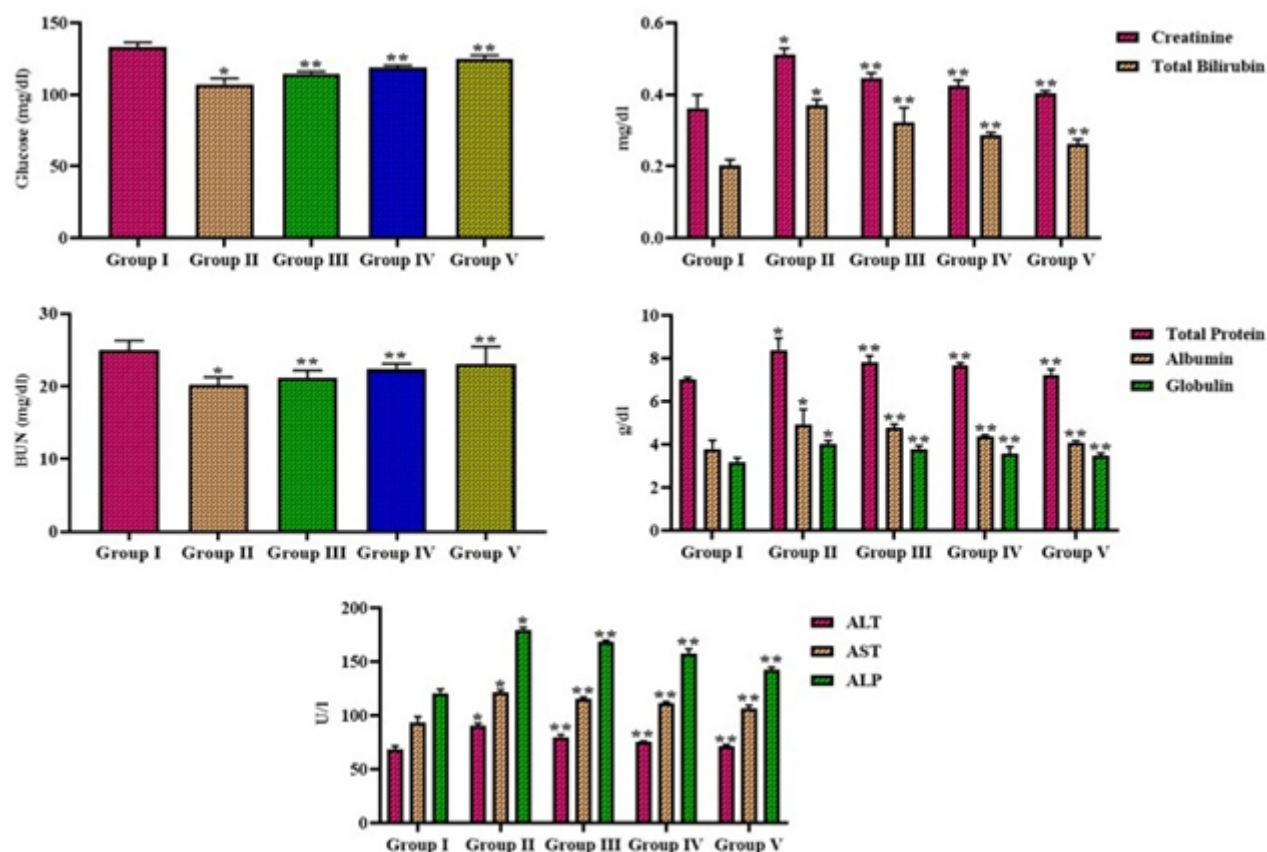


**Figure 2:** Effect of thymoquinone on the biochemical marker levels in the serum of experimental rats. The data are shown as a Mean ± SD of three replicate measurements. The values are subjected to statistical analysis by one-way ANOVA and Tukey's *post hoc* tests using Graphpad Prism. Note: '\*' specifies that the values are significant at  $p < 0.01$  in comparison to the control group; '\*\*' specifies that the values are significant at  $p < 0.05$  in comparison to the osteoarthritis-induced group.

a significant contributor to the pathophysiology of osteoarthritis, influencing various aspects of disease initiation and progression. Alterations in the redox state within the joint microenvironment can disrupt cellular function, promote inflammation, and accelerate cartilage degradation.<sup>23</sup> Oxidative stress plays a critical role in the onset of osteoarthritis by disrupting cellular homeostasis and enhancing inflammation within the joint. ROS are accumulated as byproducts of cellular metabolism and in response to external stimuli like mechanical stress, inflammatory markers, and matrix degradation products.<sup>24</sup> In osteoarthritis, the over accumulation of ROS overwhelms the antioxidant mechanisms, resulting in oxidative injury to the cellular components. The resulting oxidative modifications can impair cellular function, trigger inflammatory signaling pathways, and promote apoptosis of chondrocytes and synovial cells.<sup>25</sup>

MDA is a product of lipid peroxidation, serves as a marker of oxidative damage to cell membranes and lipoproteins. Increased MDA levels have been found in the synovial fluid and cartilage of patients with osteoarthritis, indicating elevated oxidative stress within the joint. The elevated MDA levels reflect the enhanced lipid peroxidation and oxidative damage occurring in the joint tissues, contributing to cartilage degradation and inflammation.<sup>26</sup> SOD, CAT, and GPx are antioxidant enzymes

that plays a critical role in scavenging ROS and guarding cells from oxidative injury. These enzymes work in concert to sustain redox homeostasis and prevent the accumulation of ROS within the joint. However, in osteoarthritis, the activity and expression of these antioxidant enzymes are often impaired, leading to a diminished capacity to neutralize ROS and protect against oxidative stress.<sup>27</sup> Reduced activity of these antioxidant enzymes render the joint tissues more susceptible to oxidative injury and accelerate the osteoarthritis. Targeting oxidative stress and its associated biomarkers represents a hopeful therapeutic technique for managing osteoarthritis.<sup>28</sup> The findings of this study exhibited that rats with osteoarthritis demonstrated increased MDA levels, while decreased CAT, GPx, and SOD levels when compared with control. Interestingly, the thymoquinone treatment reduced the MDA levels and subsequently elevated the antioxidant concentrations in the osteoarthritis-induced rats. These findings witness the antioxidant effects of the thymoquinone.

Inflammation is now considered a key player in the onset of osteoarthritis, with inflammatory mediators orchestrating a cascade of events that promote cartilage destruction and synovial inflammation. The inflammation in osteoarthritis is marked by heightened synthesis of pro-inflammatory markers in the joint microenvironment.<sup>29</sup> Cytokines such as TNF- $\alpha$  and IL-1 $\beta$  are key

drivers of inflammation in osteoarthritis, influencing chondrocytes, synoviocytes, and other joint tissues. These cytokines initiate signaling pathways that amplify the inflammatory response, leading to the activation of catabolic enzymes, the suppression of anabolic processes, and the sensitization of nociceptors, which contribute to pain. The sustained presence of these inflammatory mediators perpetuates a cycle of destruction within the joint, driving disease progression. The inflammatory processes in osteoarthritis are not solely confined to the cartilage but extend to the synovium, the membrane lining the joint capsule.<sup>30</sup> Synovitis, or inflammation of the synovium, is a common feature of osteoarthritis and contributes significantly to pain and swelling. The interplay between inflammation and joint tissue degradation highlights the complexity of osteoarthritis pathophysiology and suggests that therapeutic strategies targeting inflammatory mediators may have disease-modifying potential.<sup>31</sup>

TNF- $\alpha$  exerts multifaceted effects on joint tissues, particularly chondrocytes and synoviocytes, in the context of osteoarthritis. TNF- $\alpha$  stimulates the secretion of Matrix Metalloproteinases (MMPs) that degrade collagen and proteoglycans, the crucial components of the cartilage extracellular matrix.<sup>32</sup> The resultant loss of matrix integrity compromises the structural integrity of cartilage, leading to erosion and fibrillation. In addition to its influence on cartilage degradation, TNF- $\alpha$  also enhances the accumulation of other pro-inflammatory markers and amplifying the inflammatory cascade within the joint.<sup>33</sup> IL-1 $\beta$  is a master regulator of inflammation and catabolism in osteoarthritis, wielding potent effects on chondrocytes, synoviocytes, and other joint tissues. IL-1 $\beta$  stimulates chondrocytes to produce MMPs while

simultaneously blocking the secretion of collagen, key ingredient of the cartilage matrix.<sup>34</sup> This dual action promotes cartilage degradation and impairs the capacity of chondrocytes to repair or regenerate the damaged matrix. Furthermore, IL-1 $\beta$  induces the secretion of other pro-inflammatory markers, contributing to the enhancement of the inflammatory reaction within the joint.<sup>35</sup> IL-6 is a multifunctional cytokine that exerts diverse effects on immune cells, bone metabolism, and cartilage homeostasis in the context of osteoarthritis. IL-6 enhances synovitis and joint degradation by triggering neutrophil influx and osteoclast maturation, and pannus proliferation. IL-6 trans-signaling has been participated in the onset of arthritis.<sup>36</sup> IL-18, a member of the IL-1 superfamily, is participated in the inflammatory and destructive processes characteristic of osteoarthritis. IL-18 may synergize with other cytokines, like IL-1 $\beta$  and TNF- $\alpha$ , to promote inflammation and cartilage degradation in osteoarthritis. The involvement of these cytokines suggests potential therapeutic targets for managing osteoarthritis.<sup>37</sup> The present study observed the increased TNF- $\alpha$ , IL-6, IL-18, and IL-18 concentrations in the serum of MIA-induced rats. Whereas, the thymoquinone treatment considerably decreased these cytokine concentrations in the serum of osteoarthritis-induced rats, which evidences its anti-inflammatory efficiency.

The degradation of the collagen network within the cartilage is an essential event driving the development of osteoarthritis. The imbalance arises from a complex interplay of biochemical and mechanical factors that compromise the structural integrity of the joint. Chondrocyte apoptosis is considered a primary cause of the progression of osteoarthritis. This process

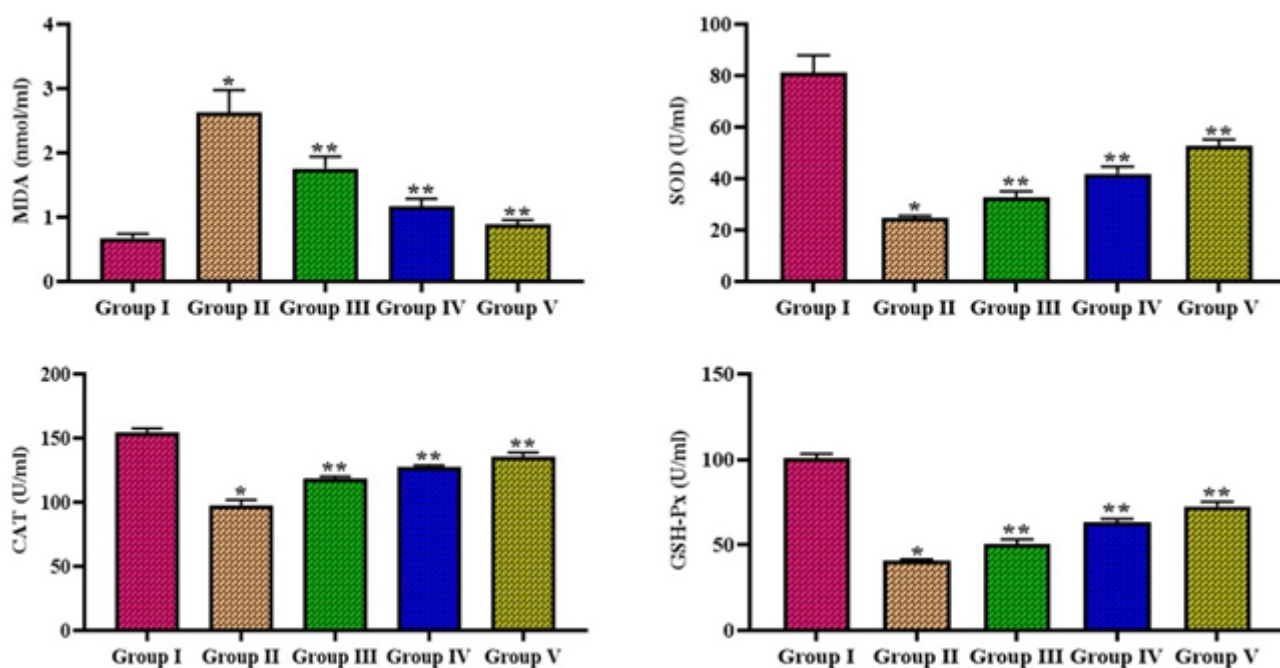


**Figure 3:** Effect of thymoquinone on the oxidative stress markers in the experimental rats. The data are shown as a Mean  $\pm$  SD of three replicate measurements. The values are subjected to statistical analysis by one-way ANOVA and Tukey's *post hoc* tests using Graphpad Prism. Note: '\*' specifies that the values are significant at  $p < 0.01$  in comparison to the control group; '\*\*' specifies that the values are significant at  $p < 0.05$  in comparison to the osteoarthritis-induced group.

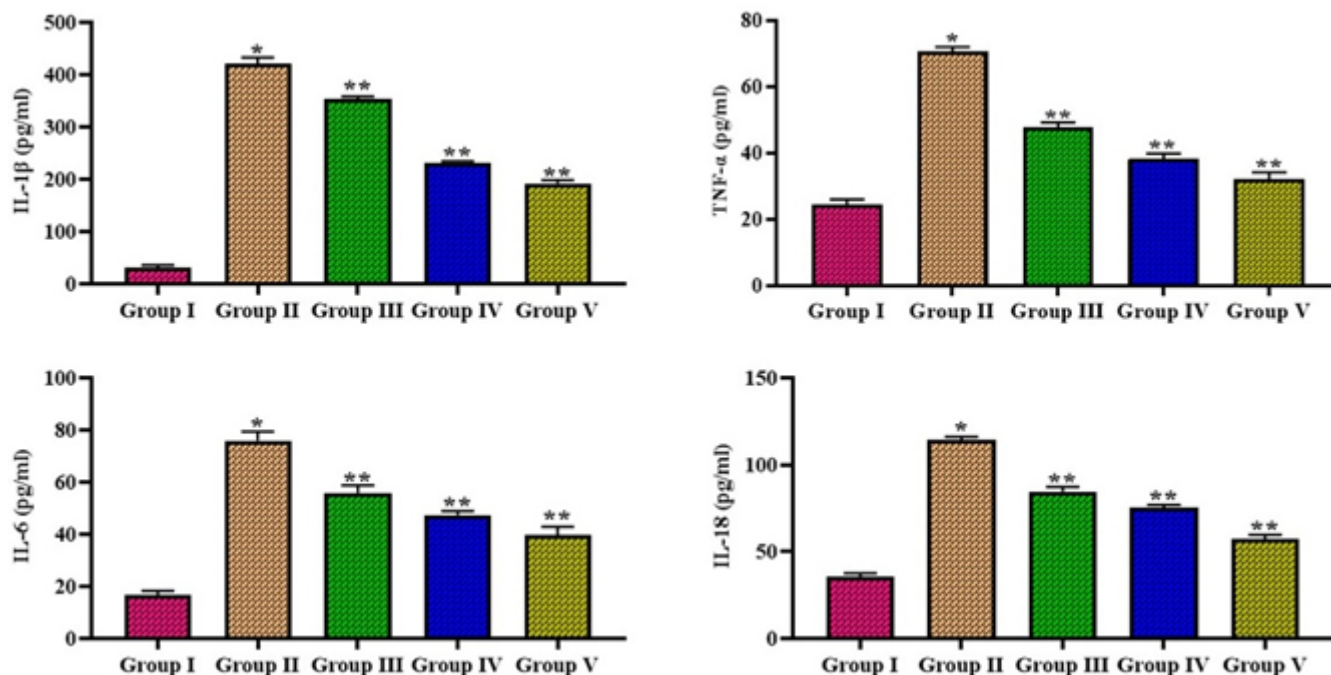


**Figure 4:** Effect of thymoquinone on the inflammatory cytokines in the experimental rats. The data are shown as a Mean±SD of three replicate measurements. The values are subjected to statistical analysis by one-way ANOVA and Tukey's *post hoc* tests using Graphpad Prism. Note: '\*' specifies that the values are significant at  $p < 0.01$  in comparison to the control group; '\*\*' specifies that the values are significant at  $p < 0.05$  in comparison to the osteoarthritis-induced group.

involves intricate molecular pathways, with caspase-3, and caspase-9 emerging as key players.<sup>38</sup> The current understanding acknowledges osteoarthritis as a multifaceted pathology influenced by inflammatory and metabolic factors that lead to joint damage. Inflammation is sustained during the progression of osteoarthritis, which contributes to the alteration of the biomechanical environment of chondrocytes. The role of PGE2 and caspase-3/9 in the pathophysiology of osteoarthritis is multifaceted, involving inflammatory signaling, chondrocyte apoptosis, and cartilage destruction.<sup>39</sup> PGE2, a lipid mediator derived from arachidonic acid, is synthesized by cyclooxygenase enzymes and PGE synthases and has emerged as an essential mediator in the pathophysiology of osteoarthritis, influencing inflammation, cartilage degradation, and pain sensitization within the affected joint. Its elevated levels in the synovial fluid and cartilage of osteoarthritic joints correlate with disease severity, highlighting its significance as a potential therapeutic target.<sup>40</sup>

Caspase-3, an effector caspase downstream of multiple apoptotic pathways, executes programmed cell death by cleaving a wide array of intracellular substrates, leading to the dismantling of cellular structures, and is a critical mediator of chondrocyte apoptosis in osteoarthritis.<sup>41</sup> Chondrocyte apoptosis is a hallmark feature of osteoarthritis, contributing to the depletion of the cartilage matrix and joint degeneration. The activation of caspase-3 in chondrocytes triggers a cascade of events that dismantle the cellular machinery, resulting in apoptosis and subsequent matrix destruction.<sup>42</sup> Caspase-9, an initiator caspase residing upstream of caspase-3 in the intrinsic apoptotic pathway,

plays an essential role in initiating apoptosis in response to mitochondrial dysfunction. Caspase-9 activation is primarily triggered by the discharge of cytochrome-c from mitochondria into the cytoplasm.<sup>43</sup> The apoptotic cascade initiated by caspase-9 activation contributes to the depletion of chondrocytes and the progression of cartilage degeneration.<sup>44</sup> In this work, we found the significant increase in the PGE2 and caspase-3/9 levels in the serum of MIA-induced rats. However, the PGE2 and caspase-3/9 were significantly reduced by the thymoquinone in the osteoarthritis-induced rats.

Nrf2, a crucial transcription factor, orchestrates the cellular defense against oxidative stress by activating the antioxidant enzyme expressions and cytoprotective proteins. Nrf2, under normal physiological circumstances, remains sequestered in the cytoplasm through its interaction with Keap1, a repressor protein that enables its ubiquitination and subsequent proteasomal degradation.<sup>45</sup> Upon exposure to oxidative stress, Nrf2 dissociates from Keap1, translocates to nucleus, and binds to antioxidant elements, beginning the transcription of genes that encode antioxidants like HO1, SOD, and CAT. The activation of Nrf2 signaling may suppress the secretion of pro-inflammatory markers and COX-2. HO1, an inducible enzyme regulated by Nrf2, facilitates the conversion of heme into biliverdin, carbon monoxide, and iron, therefore imparting significant anti-inflammatory, antioxidant, and cytoprotective attributes. The induction of HO-1 by Nrf2 contributes to the resolution of inflammation and the maintenance of tissue homeostasis in the osteoarthritic joint.<sup>46</sup>

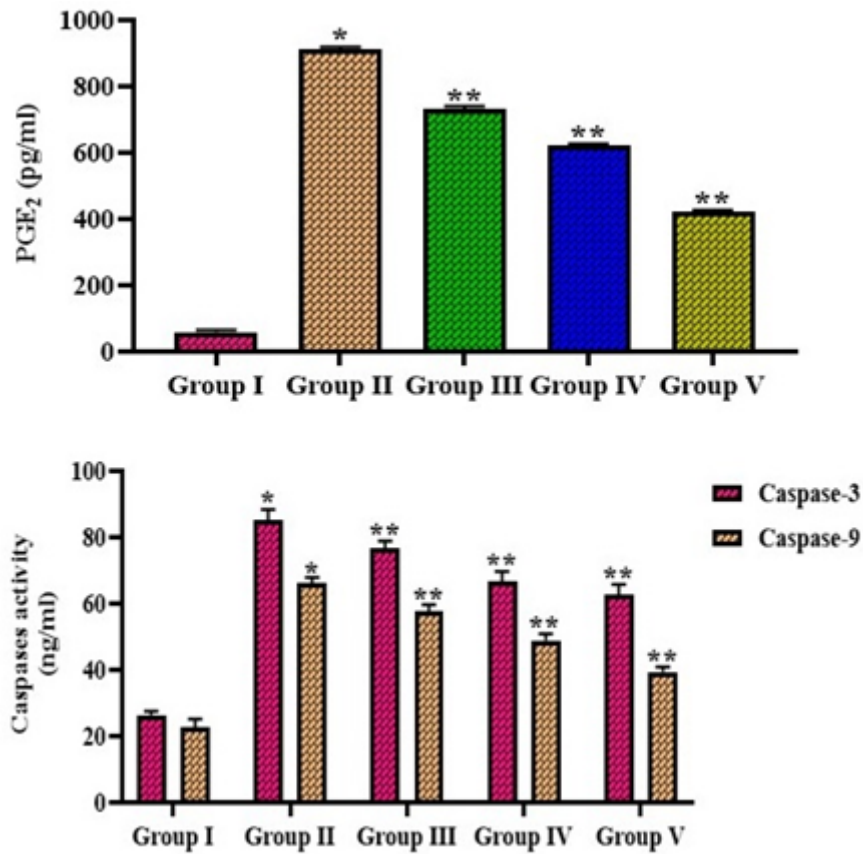


**Figure 5:** Effect of thymoquinone on the PGE<sub>2</sub> and caspase-3/9 levels in the serum of the experimental rats. The data are shown as a Mean±SD of three replicate measurements. The values are subjected to statistical analysis by one-way ANOVA and Tukey's *post hoc* tests using Graphpad Prism. Note: '\*' specifies that the values are significant at  $p < 0.01$  in comparison to the control group; '\*\*' specifies that the values are significant at  $p < 0.05$  in comparison to the osteoarthritis-induced group.

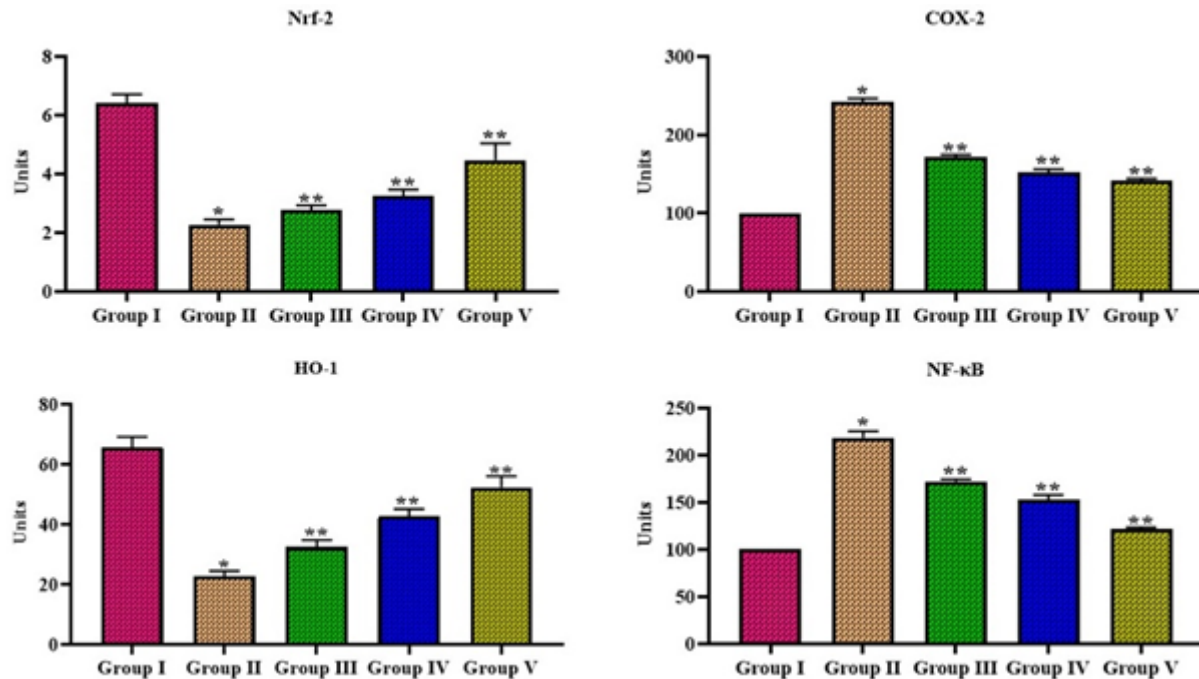


**Figure 6:** Effect of thymoquinone on the Nrf2/HO1, COX-2, and NF-κB levels in the experimental rats. The data are shown as a mean±SD of three replicate measurements. The values are subjected to statistical analysis by one-way ANOVA and Tukey's *post hoc* tests using Graphpad Prism. Note: '\*' specifies that the values are significant at  $p < 0.01$  in comparison to the control group; '\*\*' specifies that the values are significant at  $p < 0.05$  in comparison to the osteoarthritis-induced group.

NF- $\kappa$ B, a pivotal transcription factor governing the gene expressions participated in inflammation, immunity, and cell survival, is activated in response to diverse stimuli like inflammatory markers. Upon activation, NF- $\kappa$ B translocates to the nucleus and binds to specific DNA sequences, thereby promoting the transcription of pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , which exacerbate cartilage destruction and synovial inflammation in osteoarthritis.<sup>47</sup> The NF- $\kappa$ B signaling plays an essential role in maintaining the expression of MMPs, a family of enzymes responsible for degrading the extracellular matrix components of articular cartilage. NF- $\kappa$ B activation participates in the onset of osteoarthritis by enhancing the secretion of pro-inflammatory markers, thereby perpetuating the cycle of cartilage destruction and joint inflammation. Therefore, it plays a key role in the onset of osteoarthritis.<sup>48</sup> COX-2, an inducible enzyme responsible for the synthesis of prostaglandins, is upregulated in the synovial tissues and chondrocytes of osteoarthritic joints, contributing to pain, inflammation, and cartilage degradation. PGs, particularly PGE<sub>2</sub>, exert catabolic effects on cartilage by inhibiting the secretion of extracellular matrix components and promoting the release of MMPs. COX-2 production is stimulated by pro-inflammatory markers and growth factors via the activation of transcription factors including NF- $\kappa$ B. Targeting COX-2 has been considered as a therapeutic technique for osteoarthritis.<sup>49</sup> In this work, our findings revealed an elevation in the COX-2 and NF- $\kappa$ B and subsequent reduction in Nrf2/HO1 levels in the joint tissues of the MIA-induced rats. Fascinatingly, the thymoquinone treatment significantly reduced the COX-2 and NF- $\kappa$ B levels and subsequently elevated the Nrf2/HO1 levels in osteoarthritis-induced rats.

## CONCLUSION

The present findings evidenced that the thymoquinone mitigated the osteoarthritis-related pathological alterations induced by MIA in rats by modulating inflammation and oxidative stress-related biomarker levels. Furthermore, thymoquinone treatment activated the Nrf2/HO1 pathway, decreased inflammatory cytokines, NF- $\kappa$ B, and COX-2 levels, thereby mitigating the oxidative stress and inflammation in the experimental rats. These data demonstrate that thymoquinone successfully ameliorated MIA-induced osteoarthritis in rats. Additionally, further studies are necessary to completely understand the therapeutic effects of thymoquinone on osteoarthritis.

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## ABBREVIATIONS

**MIA:** Monosodium iodoacetate; **PGE-2:** Prostaglandin E<sub>2</sub>; **COX-2:** Cyclooxygenase-2; **BUN:** Blood Urea Nitrogen; **ALP:** Alkaline Phosphatase; **AST:** Aspartate Transaminase; **ALT:** Alanine Transaminase; **MDA:** Malondialdehyde; **CAT:** Catalase; **GPx:** Glutathione peroxidase; **IL:** Interleukin.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ETHICAL APPROVAL

This work has approved by the institutional animal ethical committee by Jiangdu People's Hospital Affiliated to Yangzhou University, Yangzhou Jiangsu, 225200, China.

## SUMMARY

Osteoarthritis is a major global health issue, resulting in disability, reduced quality of life, and high healthcare expenditures. The intricate characteristics of osteoarthritis arises from its diverse causes, including genetic predisposition, biomechanical factors, inflammation, and metabolic irregularities. The current findings demonstrated that thymoquinone treatment alleviated the osteoarthritis-associated degenerative changes induced by MIA in rats via regulating inflammation and oxidative stress biomarkers. These findings indicate that thymoquinone effectively mitigated MIA-induced osteoarthritis in rats.

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